Observing biodegradation of complex organic substrates by site-specific isotopic analyses

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Microbial fermentation of organic substrates substrates is often accompanied by kinetic isotope effects that preferentially enrich residues in ^{13}C. However, it is difficult to recognize these fractionations in natural materials both because intial abundances of the reactive moities in sedimentary organic matter (e.g., lignin, pectin, cellulose) are rarely known, and because biodegradation affects only a small fraction of sites, meaning any isotopic change is diluted by an isotopic measurement of bulk compounds. Methoxy groups ($R–O–CH_3$) are major components of the biopolymers lignin and pectin (up to 10 wt.%) and are preferentially consumed by microbial fermentation in anaerobic environments. Here, we present methods for determining the singly- and doubly-substituted site-specific isotopic compositions of methoxyl groups ($R–O–CH_3$) in natural materials, and interpret isotopic signatures associated with their loss during the diagenesis of organic matter.

Ether-bound methyl groups were extracted from woody tissues, brown coals, and humic lignites by chemical reaction with hydriodic acid to produce iodomethane (CH_3I). Extracts were converted to fluoromethane (CH_3F) on cobalt trifluoride, cryogenically purified, and measured for $\delta^{13}C$, $\delta^{2}H$, and clumped $^{13}C^{2}H$ composition by high-resolution dual-inlet IRMS. The $\delta^{13}C$ values of methoxy groups in undegraded woody tissues are similar to those of bulk organic carbon, but in more degraded materials are dramatically ^{13}C enriched relative to bulk $\delta^{13}C$, by up to 55‰. These extreme enrichments are observed in materials with low methoxyl group concentrations, consistent with them being residues of relatively fractionating microbially-mediated degradation with an isotope effect similar to that measured in laboratory cultures. This interpretation agrees with independent observations of indigenous microbial assemblages that have persisted while entombed in the studied sedimentary units for >10 myr. Methoxyl $\delta^{2}H$ and clumped $^{13}C–^{2}H$ values show systematic ordering associated with maturity and origin, but their undegraded counterparts are unconstrained. These data demonstrate the potential of methoxyl groups, and site-specific isotope measurements of organic materials in general, to record microbial activity in natural environments.